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EMBRYONIC DEVELOPMENT OF MELANOCYTES IN HUMAN HAIR AND EPIDERMIS

THEIR CELLULAR DIFFERENTIATION AND MELANOGENIC ACTIVITY*

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The biological and enzymic behavior of embryonic melanocytes and their cellular interaction in differentiating follicular and epidermal structures after leaving their dermal environment through the penetration of the dermal-epidermal junction has not been significantly studied, although their neural crest origin (1) and developmental process within the dermis including their epidermal entry (2) is well described.

Moreover, the melanocyte systems of adult human hair follicles excluding the infundibulum have been found to be different from that of the epidermis in their melanin synthesizing activity. The melanocyte in the peripheral layer of the outer root sheath below the melanogenic level has been described as being an "amelanotic melanocyte (3)" based on its negative reaction to dopa and Masson's premelanin technic. Furthermore, the hair bulb melanocyte has been shown to differ from the epidermal melanocyte in being not only dopa but also tyrosinase reaction positive in the anagen stage of the follicle (4).

The origin and developmental genesis of these distinctive hair and epidermal melanocyte systems, however, have not been studied in relation to embryonic hair and epidermal differentiation despite its biological importance. Previous embryonic studies were mostly morphological characterizations which did not present information on the melanogenic activity of these developing melanocytes. In the present study the cellular differentiation, tyrosinase synthesis, and the formation of premelanin positive melanosomes and their melanization have been investigated in the melanocytes of developing embryonic human hair follicles and epidermis.

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MATERIALS AND METHODS

Skin specimens of various areas were taken from 72 Japanese fetuses, of which ten cases were found to have a sufficiently large number of hair follicles in various stages of development in order to make the statistical analysis shown in Table 1. The ten cases finally selected consisted of three months to eight months old fetal scalp except for one four month leg skin (F-69-III), one six month breast skin (F-31) and one six month genital skin (F-44).

The specimens were studied with paraffin dopa reaction, combined dopa-premelanin reaction (5) and the ammoniated silver nitrate reaction for premelanin (5, 6). Additionally the ten micron thick paraffin sections were stained with periodic acid Schiff reaction with Alcian blue counter-stain, toluidine blue, orcein-Giemsa (7), and hematoxylin and eosin.

The cellular classification of the melanoblast and melanocyte is used according to that of Zimmermann and Becker (2).

RESULTS

In our study the developmental stages of the embryonic human hair follicle were divided according to Pinkus' description and classification (8) into pre-germ, hair germ, hair peg and bulbous peg stages. The salient findings are listed in Table 1.

I. The Melanocyte and Its Melanogenic Activity in the Pre-Germ Stage

In the pre-germ a crowding of nuclei (Fig. 1) is seen as the first sign of hair follicle development in the basal layer of the epidermis (8). Melanocytes are scattered throughout the epidermis and pre-germ follicles without any higher concentration appearing in the hair follicle at this stage of development (Fig. 1, 2). The pre-germ stage is usually observed in fetuses of three or four months age in which the epidermis shows in addition to junctional melanocytes a large number of high level melanocytes. In a number of areas these high level melanocytes, some of which freely extend highly elongated dendrites parallel to the basal layer, appear in greater numbers than those of the junction layer in the immature epidermis

TABLE 1A

Embryonic development of active and inactive melanocyte systems in human hair and epidermis

Stage			Pre-germ				Hair Germ								Hair Peg					
Case No. (Age)			F60-I (3M + 1W)		F 1 (4M)	F69-III (4M)	F60-I (3M + 1W)		F69-III (4M)	F65-II (5M + 1W)	F70-I (5M)		F70-II (5M)	F44 (6M)	F60-I (3M)		F70-I (5M)		F70-II (5M)	F44 (6M)
Reactions			CDP	Dopa	Prem	Prem	CDP	Dopa	Prem	Prem	CDP	Dopa	Prem	Prem	CDP	Dopa	CDP	Dopa	Prem	Prem
Hair Follicle	CELL	INNER	+	−	+	+	++ 60%	+ 10%	++ 33%	++ 60%	++ 60%	+ 50%	++ 40%	++ 80%	++ 90%	+ 50%	++ 90%	+ 90%	++ 70%	++ 100%
		PERIPHERAL	33%	0%	33%	33%														
	LAYER						++ 60%	+ 10%	++ 33%	++ 50%	++ 50%	+ 50%	++ 50%	++ 80%	++ 90%	+ 50%	++ 90%	+ 80%	++ 80%	++ 100%
EPIDERMIS	High Level Layer		++	+	+	++	++	+	++	+	+	+	+	−	++	+	+	+	+	+
	Junction Layer		++	+	++	+	++	+	+	++	++	+	++	++	++	+	++	+	++	++

CDP: Combined Dopa-Premelanin Reaction (5).
Prem: Ammoniated Silver Nitrate Reaction for Premelanin (6).
% number: Indicates the percentage of follicles exhibiting the degree of positivity shown.
(+)-(+++): Represents difference in number of melanocytes revealed by reaction in individual follicles.
(−): No melanocytes revealed by reaction.

TABLE 1B

Embryonic development of active and inactive melanocyte systems in human hair and epidermis

Stage			Bulbous Peg													
Case No. (Age)			F65-I (5M + 1W)		F65-II (5M + 1W)	F70-I (5M)		F70-II (5M)	F31 (6M)	F3 (7M)	F48-I (8M)	F48-II (8M)		F49-I (8M)	F48-II (8M)	
Reactions			CDP	Dopa	Prem	CDP	Dopa	Prem	Prem	Prem	Prem	CDP	Dopa	Prem	CDP	Dopa
Hair Follicle	CELL LAYER INNER	A	+10%	–	+10%	+10%	–	+20%	–	–	–	–	–	–	–	–
		B	+20%	–	–	–	–	–	–	–	–	–	–	–	–	–
		Ca	+10%	–	+10%	–	–	+10%	–	–	–	–	–	–	–	–
		Cb	++90%	+10%	++70%	++90%	+20%	++70%	++60%	+50%	++80%	++70%	+50%	++80%	++80%	+70%
	CELL LAYER PERIPHERAL	A	++100%	–	++100%	++100%	+100%	++100%	++100%	++100%	++100%	++100%	+100%	++100%	++100%	+100%
		B	+10%	–	+10%	+20%	–	+10%	+10%	–	–	–	–	–	–	–
		Ca	+20%	–	+10%	–	–	+20%	–	–	–	–	–	–	–	–
		Cb	++70%	+10%	++80%	++70%	+10%	++60%	++50%	+50%	++100%	++90%	+50%	++100%	++100%	+60%
	D		+++ 100%	+ 100%	+++ 100%	+++ 100%	++ 100%	+++ 100%	+++ 100%	+++ 100%	+++ 100%	+++ 100%	++ 100%	+++ 100%	+++ 100%	++ 100%
EPIDERMIS	High Level Layer		±	+	+	+	+	+	–	–	–	–	–	–	–	–
	Junction Layer		++	+	++	++	+	++	++	++	++	++	+	++	++	+

Bulbous peg is divided into the following portions (Figs. 14, 15): A: Infundibulum; B: Middle; Ca: Upper Bulb; Cb: Lower Bulb; D: Pigment Matrix.

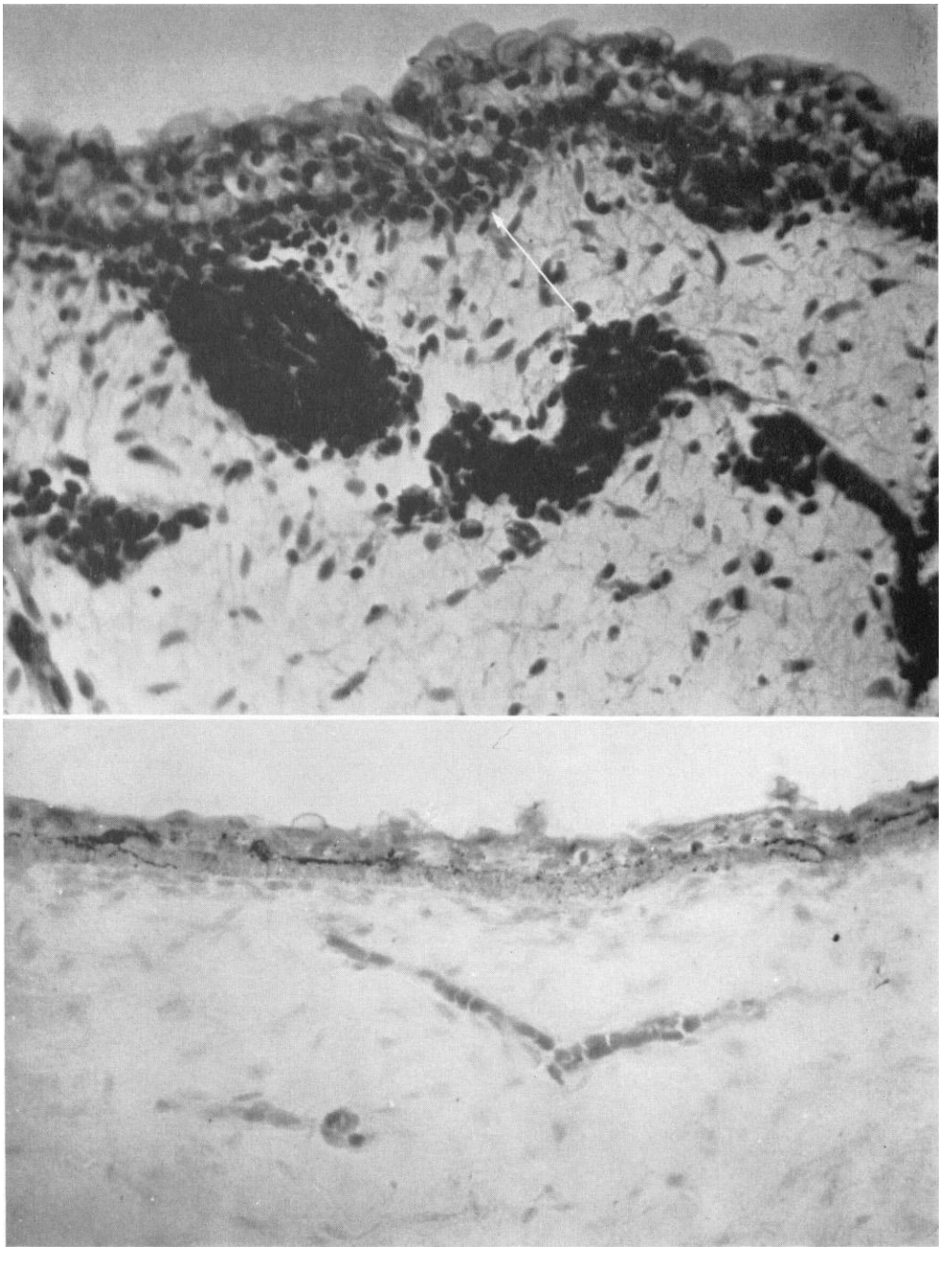

FIG. 1. Three month old human fetus showing a melanocyte in pregerm (arrow) stage of hair follicle development formed by a crowding of nuclei in the basal layer of the epidermis. Combined dopa-premelanin reaction counterstained with orcein-Giemsa, F60-I (scalp skin). (465 ×.)

FIG. 2. Four month old human fetus revealing scattered bipolar melanocytes in the high-level layers of the epidermis. Premelanin reaction. F69-III (leg skin). (465 ×.)

of two or three layers (Fig. 2, 3). The melanocytes in this stage are predominantly bipolar while some appear polydendritic. The above melanocytes and also the ones discussed below are premelanin reaction and dopa reaction positive although the premelanin reaction reveals a definitely larger number of melanocytes than the dopa reaction. No melanoblast, which by definitition (2) is a round or ovoid premelanin reaction positive dopa negative cell, is observed in the hair follicle or epidermis throughout their development.

II. The Melanocyte and Its Melanogenic Activity in the Hair Germ Stage

In this stage the basal cells become high and columnar, the nuclei elongate and the follicle extends downward into the dermis (8). At this stage there is still no higher concentration of melanocytes in the hair germ. The horizontal distribution of melanocytes is almost uniform throughout the epidermis and hair germ (Fig. 4). The vertical distribution of melanocytes in the hair germ is almost random between the peripheral and inner cell layers of the germ (Fig. 5). This stage of follicle development is observed from three to six months in which the epidermis continues to show a number of high level melanocytes containing premelanin and dopa reaction positive cytoplasmic granules (Fig. 6) although the epidermal melanocytes begin to exhibit a tendency of junctional localization (Fig. 5). This wide range in age for certain developmental stages of embryonic hair is due to the fact that with continued growth of the skin, in addition to secondary hair germs (Fig. 7), later primary hair follicles develop between the earlier primary hair follicles when the distance between the early primary follicles become widely separated and a critical distance reached (8). Thus fetal skin contains follicles in more than one stage of development and some specimens from a younger fetus show more advanced hair development than specimens from an older one. This made it necessary to describe the findings of hair follicle melanocyte development according to the stage of individual follicles as used in our tables rather than in simple chronological order. On the other hand this non-chronological division raises some difficulties for the description of the epidermal melanocyte development which theoretically requires a division according to age.

However, the overall picture reveals that the epidermal melanocyte has a continuous developmental direction: from a randomly organized state towards normal permanent localization. Furthermore this continuous development is generally parallel both to the follicular stage and age. Therefore in the embryolcgy of the melanocyte in relation to hair follicles and epidermis the stage of the hair follicle and the age of the fetus are both considered in the description of our findings.

III. The Melanocyte and its Melanogenic Activity in the Hair Peg Stage

During this stage of development the hair follicle descends obliquely into the dermis in the shape of a solid column of epithelial cells (8). The melanogenic activity in this stage is similar to that of the hair germ stage. According to Danneel and Weissenfels epidermal melanocytes migrate downward through the "external root sheath" (17). However, dopa and premelanin reaction positive melanocytes continue to be distributed almost randomly throughout the peripheral and inner cell layers of the follicle although the epidermis continues to show a tendency of melanocyte localization at the junction layer (Fig. 5). Even the hair bulb anlage at the tip of the hair peg shows no higher concentration of melanocytes. The number of hair follicles which appear to contain more dopa and premelanin reaction positive melanocytes, increases over that of previous stages although the actual population density of these melanocytes seems not to be raised. This is due to the fact that the follicle is getting larger and becoming composed of more cells which are tightly packed together in a more organized pattern.

IV. The Melanocyte and Its Melanogenic Activity in the Bulbous Peg Stage

In this stage of embryonic hair development the infundibulum, sebaceous gland, bulge, hair bulb, and pigment matrix are beginning to differentiate (8). This type of embryonic hair follicle which is similar in appearance to the adult follicle and is observed from five months fetal age to the end of embryonic development may be divided into the following areas (3):

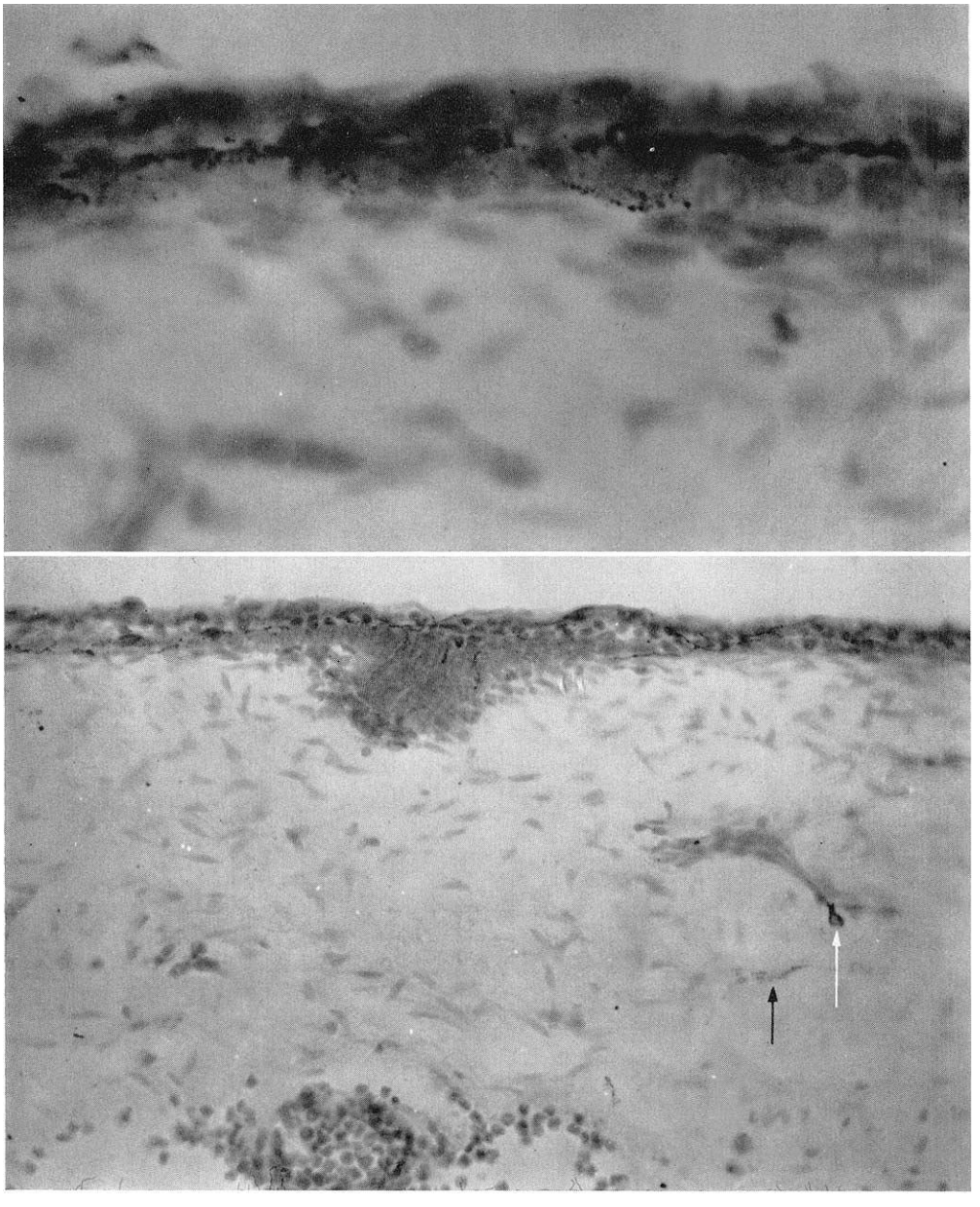

FIG. 3. High level melanocytes extending unique elongated dendrites parallel to the basal layer. Premelanin reaction. F69-III (four months leg skin). (1100 ×.)

FIG. 4. Hair germ stage demonstrating random horizontal distribution of melanocytes throughout the epidermis without a higher concentration appearing in the hair germ. In the dermis a melanoblast (white arrow) and a bipolar dendritic immature melanocyte (black arrow) are seen. Premelanin reaction. F69-III (four months leg skin). (370 ×.)

portion A the infundibulum, portion B the part of the follicle between the infundibulum and the hair bulb, portion C the hair bulb and portion D the pigment matrix (Figs. 14, 15). In this study the bulb has been divided into portions Ca above the "critical level" described by Auber (9) and Cb below this level. The designation "critical level" refers to a line drawn

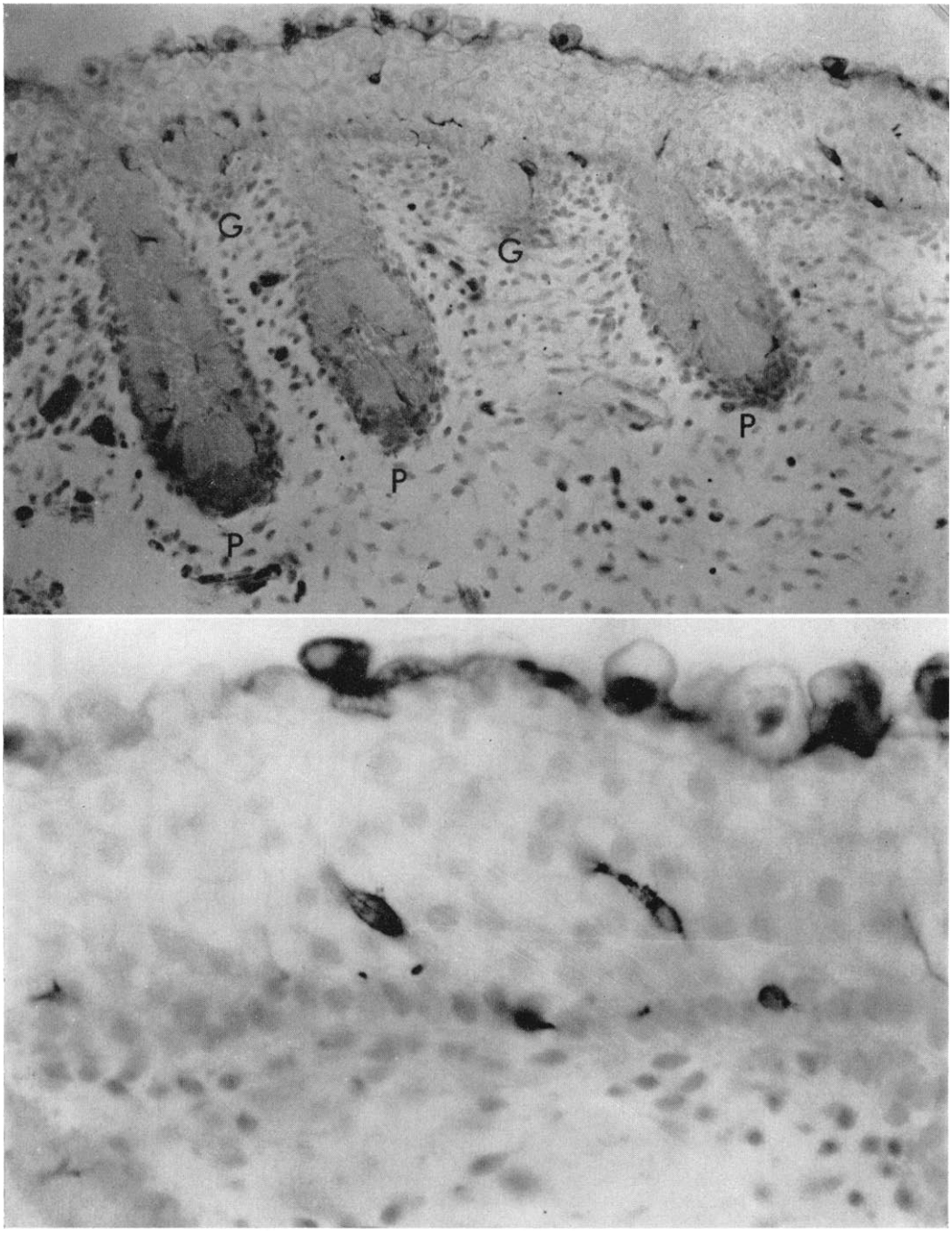

FIG. 5. The distribution of melanocytes is almost at random in both the hair germs (G) and hair pegs (P) although the epidermal melanocytes begin to exhibit a tendency of junctional localization and the hair peg melanocytes show occasionally some tendency for localization along the border between the outer columnar cell layer and the inner portion. Combined dopa-premelanin reaction. F60-I (three months scalp skin). (329 ×.)

FIG. 6. Closer view of Fig. 5 showing high level and junctional embryonic melanocytes revealing distinct discrete cytoplasmic granules which are premelanin reaction positive characteristic of melanosomes (6). Combined dopa-premelanin reaction. F60-I (three months scalp skin). (979 ×.)

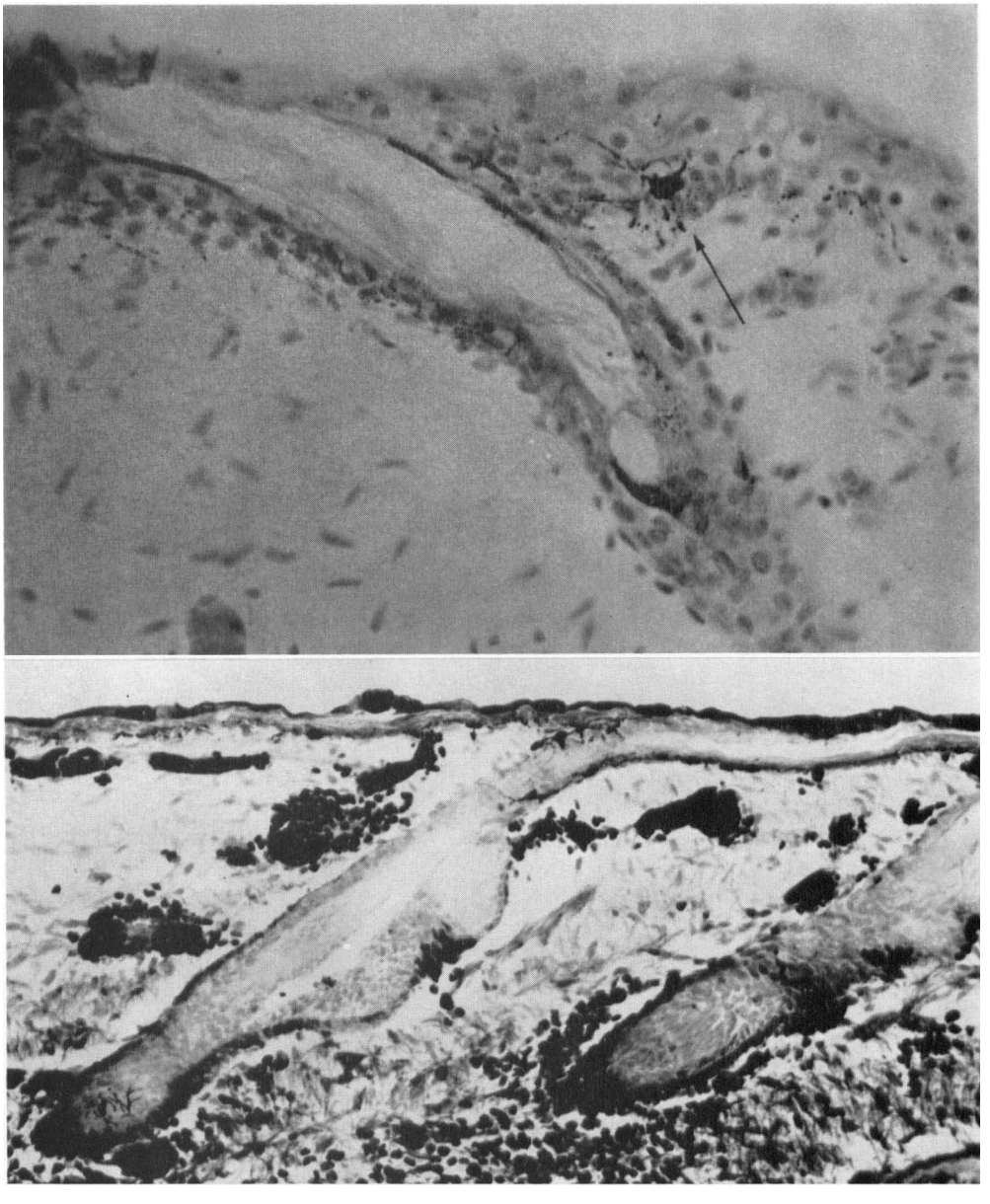

FIG. 7. Five months old human fetal scalp showing a secondary hair germ in hair germ stage (arrow) and a primary in bulbous peg stage. A premelanin positive dendritic melanocyte is seen in the secondary germ. Premelanin reaction. F70-II. (465 ×.)

FIG. 8. Bulbous peg stage hair follicle showing localized distribution of melanocytes in the peripheral layer of the outer root sheath as an extension of the junctional localization of epidermal melanocytes. Combined dopa-premelanin. F70-I (five months fetal scalp). (225 ×.)

through the widest part of the follicular dermal papilla, not necessarily the widest portion of the bulb. This line separates approximately the germinative center of the follicle (matrix) below from the differentiating cells (upper bulb) of the hair and root sheath above. In adult human hair follicles melanotic melanocytes of the hair bulb are localized above the critical

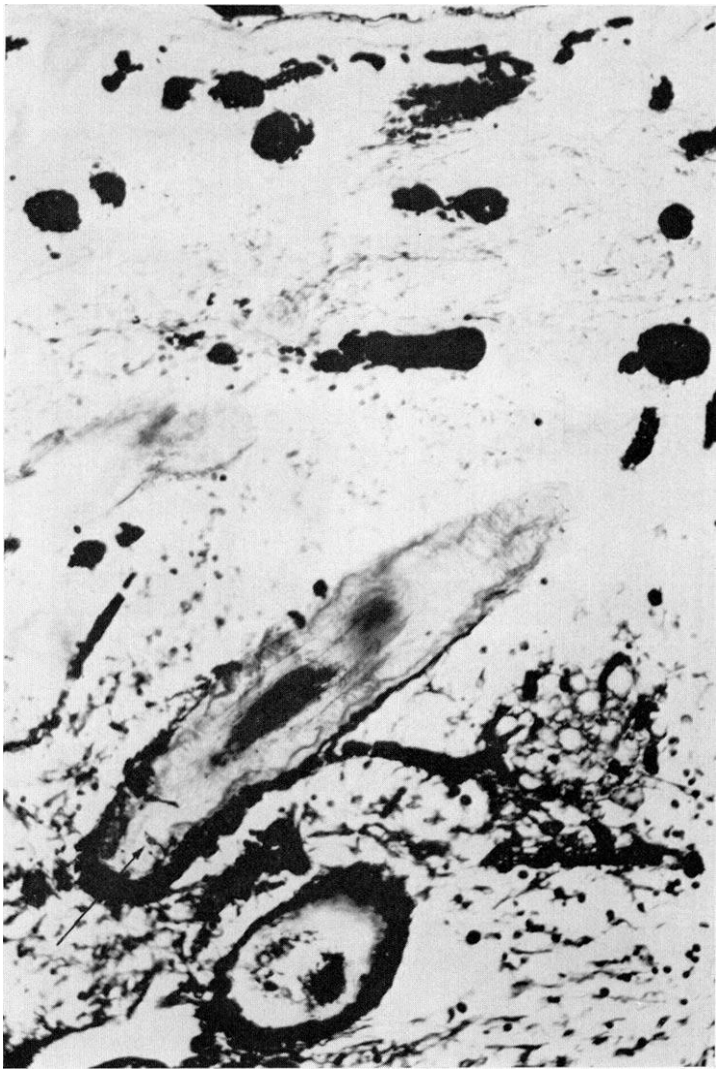

FIG. 9. Bulbous peg stage hair follicle in sixth fetal month exhibiting a premelanin positive melanocyte (arrow) in the peripheral layer of the outer root sheath of portion B (middle area). Premelanin reaction. F-31 (160 ×.)

level adjacent to the dermal papilla, supplying pigment granules for hair color. This area (portion D) is therefore designated "pigment matrix" in this study.

In portion A (infundibulum) dopa reaction and premelanin reaction positive melanocytes are almost completely localized in the peripheral layer of the outer root sheath at five months fetal age as a continuation of the localized distribution of the junction layer melanocytes (Fig. 8). Premelanin reaction positive melanocytes appear very infrequently in the inner cell layers of this part of the follicle up to six months fetal age, although no distinct dopa reaction positive melanocytes could be observed. However, after six months fetal age, no melanocytes are observed by either premelanin or dopa reaction in the inner cell layers and the melanocytes in this area of the follicle are thus exclusively localized in the peripheral layer.

In portion B (middle area) no distinctive dopa reaction positive melanocytes have been observed. However, premelanin reaction positive melanocytes are seen occasionally in the

FIG. 10. Bulbous peg stage hair follicle showing melanocytes (arrow) in the inner root sheath of portion B (middle area). Combined dopa-premelanin reaction. F65-I (five months scalp skin). (227 ×.)

peripheral layer of the outer root sheath (Fig. 9) and in the inner cell layers including the inner root sheath (Fig. 10) in the fetuses five and six months old. After six months fetal age no premelanin reaction positive nor dopa positive melanocytes are seen in this area.

In portion Ca (hair bulb above critical level excluding the pigment matrix) premelanin reaction positive, dopa reaction negative melanocytes are observed occasionally only in fetuses around five months old in both inner and outer cell layers (Fig. 11).

Portion Cb (hair bulb below critical level) exhibited dopa and premelanin reaction positive melanocytes in the inner and outer cell layers including both the external and papillar sides throughout the bulbous peg stage (five months to end of embryonic development). Additionally, melanotic melanocytes demonstrated by the toluidine blue stain were similarily distributed in this portion of the follicle in fetuses as young as five months old as soon as the bulb is formed by its anlage (Fig. 12).

In portion D (pigment matrix) melanotic

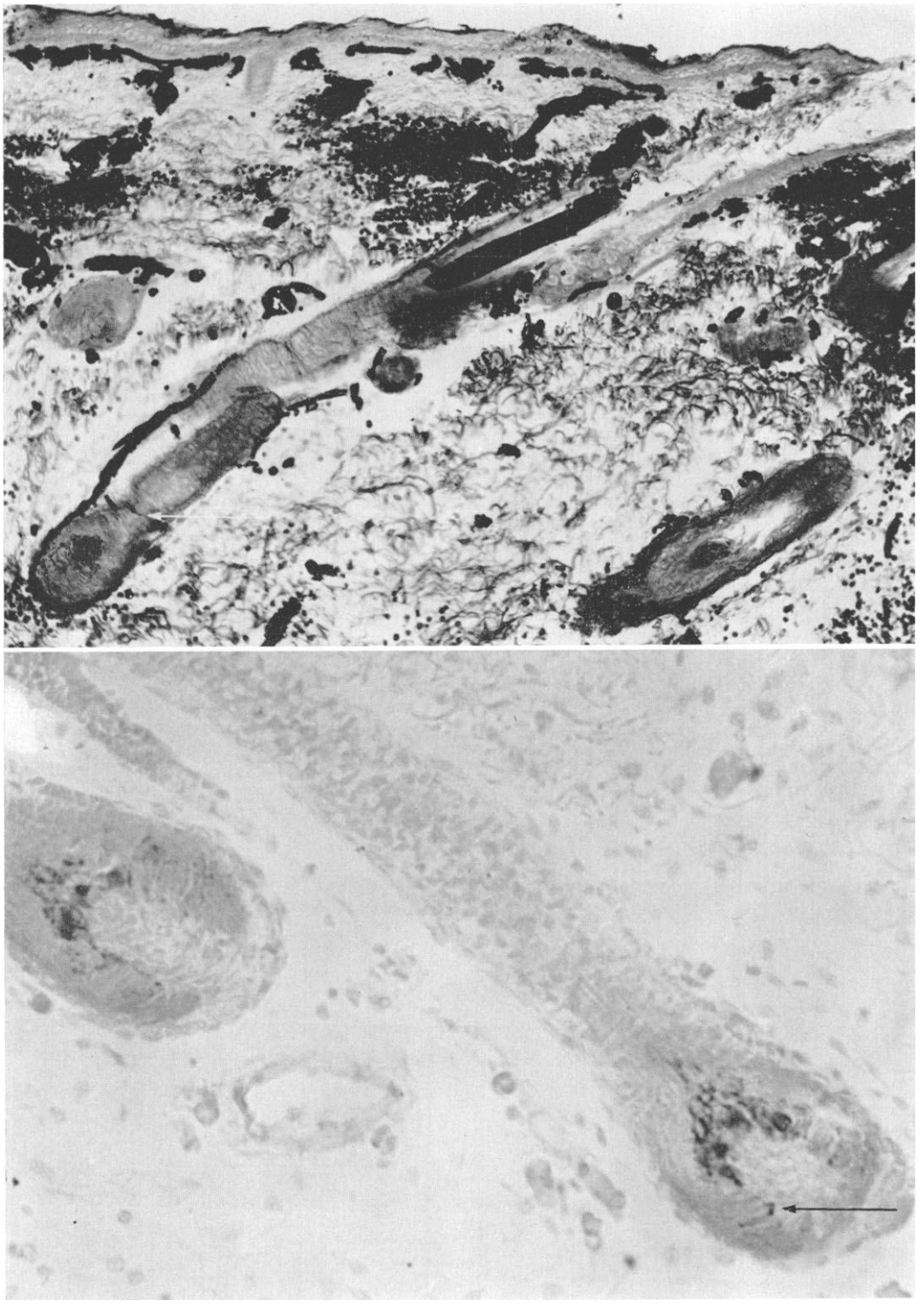

FIG. 11. Bulbous peg stage hair follicle exhibiting a melanocyte (arrow) in the outer cell layer of portion Ca (upper bulb). Combined dopa-premelanin reaction. F65-I (five months scalp skin). (162 ×.)

FIG. 12. Bulbous peg stage hair follicle showing a large number of melanotic melanocytes forming the pigment matrix above the critical level. In addition there are scattered melanotic melanocytes (arrow) below the critical level in contrast to adult conditions. Toluidine blue. F65-II (five months fetal scalp). (445 ×.)

TABLE 2

Embryonic melanocyte differentiation in human hair and epidermis

Developmental Stages	Epidermis		Hair Follicle					
	Layer	Findings	Layer	(A) Infundibulum	(B) Middle	(Ca) Upper bulb	(Cb) Lower bulb	(D) Pigment Matrix
Pre-germ	High Level	++ (+)	No Division	No Division		+	(±)	
	Junction	++ (+)						
Hair Germ	High Level	+ (+)	Inner	No Division		++	(+)	
	Junction	++ (+)	Peripheral	No Division		++	(+)	
Hair Peg	High Level	+ (+)	Inner	No Division		++	(+)	
	Junction	++ (+)	Peripheral	No Division		++	(+)	
				(A)	(B)	(Ca)	(Cb)	(D)
BULBOUS PEG Before six months	High Level	+ (+)	Inner	± (−)	± (−)	± (−)	++ (+)	+++ (++)
	Junction	++ (+)	Peripheral	++ (+)	± (−)	± (−)	++ (+)	
BULBOUS PEG After six months	High Level	− (−)	Inner	− (−)	− (−)	− (−)	++ (+)	+++ (++)
	Junction	++ (+)	Peripheral	++ (+)	− (−)	− (−)	++ (+)	

Within ( ): Dopa Reaction.

Outside ( ): Combined Dopa-premelanin or premelanin reaction.

Grading: −, absence of melanocytes; ±, a few in very occasional follicules; +, a few; ++ many; +++, very abundant. For hair follicles this grading was arrived at in the following way: number of melanocytes in individual follicles X percentage of follicles exhibiting this amount.

melanocytes which are dopa and premelanin reaction positive appear at the beginning of the bulbous peg stage and remain throughout embryonic development. The lower end of the fetal pigment matrix is, in contrast to adult conditions as described previously (8), not absolutely demarcated at the critical level and is in continuation with scattered melanocytes present in the papillar side of the lower bulb (Fig. 12).

In this stage of hair development the epidermal melanocyte pattern exhibits two substages (Table 2) similar to that of the follicular melanocytes as described above. Until the fetus becomes approximately six months old, epidermal melanocytes still appear in the high level layers retaining their premelanin and dopa positive properties although they are for the most part localized at the dermal-epidermal junction. However, after approximately six months fetal age, the epidermal melanocytes show localization limited to the dermal-epidermal junction as seen in post fetal skin. The melanocyte differentiation in embryonic human hair and epidermis as described above is summarized in Table 2 and in the schematic drawings (Figs. 13, 14, 15).

DISCUSSION

It became evident that the melanocyte differentiation and distribution in human embryonic hair follicles proceeds essentially parallel to that of the melanocyte of the embryonic epidermis (Table 2). Dopa and premelanin reaction positive melanocytes manifest their presence in embryonic hair from the earliest pre-germ stage to the end of the hair peg stage without any specific localization or higher concentration. However, this random distribution of follicular melanocytes disappears as soon as the follicle has matured into the bulbous peg

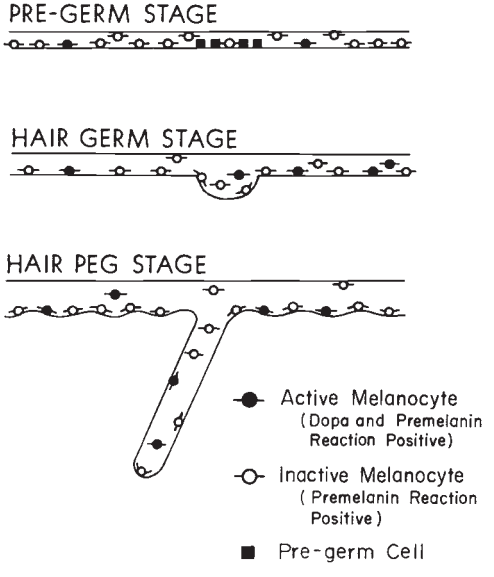

FIG. 13

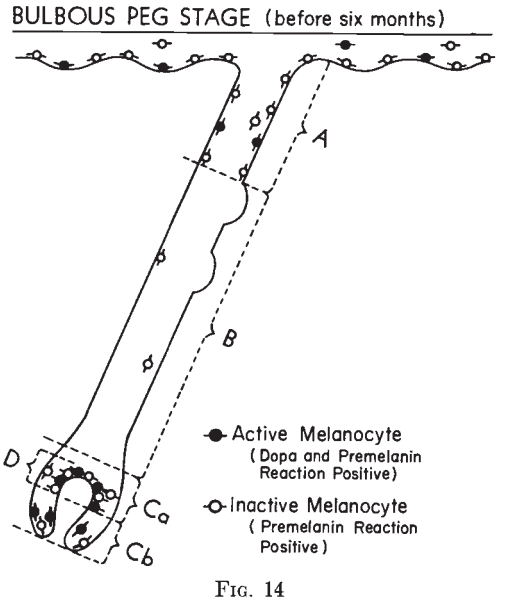

FIG. 14

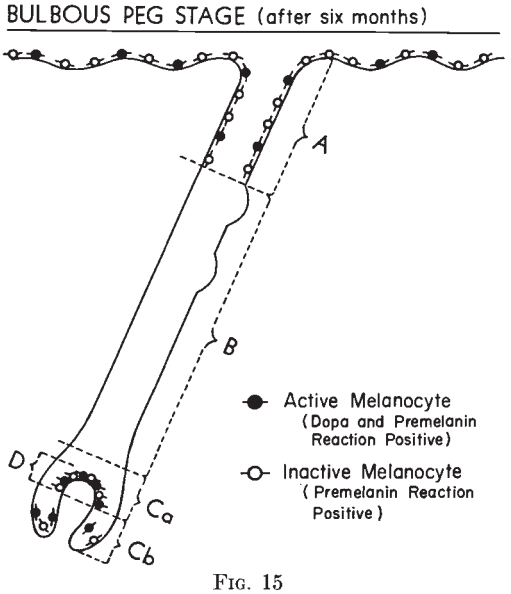

FIG. 15

stage at five months fetal age. From this time up to six months in the bulbous peg stage the vast majority of melanocytes are localized in the peripheral layer of the outer root sheath of the infundibulum (portion A), the lower bulb (portion Cb) and the pigment matrix (portion D) although a few scattered premelanin reaction positive melanocytes appear in the inner cell layers of the infundibulum, and both the inner and outer cell layers of the middle portion (portion B) and the upper bulb (portion Ca). However, after six months fetal age in the bulbous peg stage the follicular melanocytes are completely localized in the peripheral layer of the outer root sheath of the infundibulum, the lower bulb and pigment matrix as demonstrated by both the dopa and premelanin reactions.

The epidermal melanocyte also exhibits a random distribution in the early differentiating epidermis from its time of epidermal entry up to six months fetal age although the epidermal melanocyte already exhibits a distinct tendency for junctional localization at five months fetal age. After six months fetal age all epidermal melanocytes assume their permanent position at the dermal-epidermal junction as revealed by the dopa and premelanin reactions.

The dendritic cells in the high level layers of normal adult epidermis as well as in the inner cell layers of hair follicles which can be demonstrated by gold staining technics (10) and the osmium iodide method (11) but not by the dopa nor premelanin reactions have been called Langerhans cells. The origin and nature of Langerhans cells are still disputed. Electron microscopic studies show that the Langerhans cell synthesizes distinctive characteristic disc like bodies which are similar to the melanosomes of vitiligo melanocytes. Recently Breathnach and Wyllie (12) have shown in a fourteen week old fetal epidermis cells synthesizing these characteristic Langerhans cell granules not only in the high level layers but also in the basal layer of this epidermis. Our high level dendritic cells being both dopa and premelanin reaction positive and exhibiting an initial abundant presence with a gradual decrease along the fetal differentiation process indicate that the high level cells described in this study are melanocytes and not Langerhans cells. This indicates that in differentiating human fetal skin melanocytes as well as Langerhans cells cannot be defined according to their position in the epidermis since both cells exist in both the basal and high level layers.

Concerning the fate of the dopa and premelanin reaction positive high level melanocytes in fetal epidermis the available evidence suggests three possibilities existing either singly or in combination: They may be transformed into Langerhans cells; they may be drawn back to the junction; or they may be cast off from the epidermal surface.

In contrast to Zimmermann and Cornbleet's study of Negro fetuses (13) showing premelanin reaction-positive melanocytes at the time of their epidermal entry in the early part of the third month together with a faint dopa-positive reaction, Breathnach and Wyllie (12) state that their fourteen week old Caucasian fetus did not exhibit any premelanin reaction positive melanocytes, although they did contain premelanosomes. In our study of Japanese fetuses not only premelanin but also distinctly dopa-positive epidermal melanocytes are observed already at thirteen weeks fetal age.

The ammoniated silver nitrate reaction for premelanin is specific not only for the variously melanized melanosomes and melanin granules but also the cytoplasmic structures of melanosomes themselves as shown in the electron histochemistry of unmelanized melanosomes of albino melanocytes (14). We have shown in the present study, although using paraffin sections instead of fresh tissues for the premelanin reaction, that the number of melanocytes having premelanin reaction positive cytoplasmic granules exceeds the number of dopa-positive melanocytes and further we agree with Zimmermann's finding (15) that the premelanin reaction reveals embryonic melanocytes before they are dopa positive. It has been shown (16) that the formation of the characteristic cytoplasmic structure of the melanosome and the deposition of melanin within are independent although normally coordinated processes. It is therefore probable that in the fetal melanocyte differentiation the melanosome formation by the Golgi apparatus can occur prior to active tyrosinase synthesis by ribosomes.

SUMMARY

1. Embryonic differentiation of melanocytes in human fetal hair follicles and epidermis has

been studied using primarily the ammoniated silver nitrate reaction for premelanin and the dopa reaction.

2. Dopa- and premelanin-positive melanocytes appear in embryonic hair from the earliest pre-germ stage to the end of the hair peg stage without any specific localization or higher concentration.

3. In the bulbous peg stage before six months fetal age melanocytes are mostly localized in the peripheral layer of the outer root sheath of the infundibulum, the lower bulb and the pigment matrix although a few premelanin-positive melanocytes are seen in the inner cell layers of the infundibulum and in the middle portion and upper bulb.

4. After six months fetal age the bulbous peg exhibits complete localization of melanocytes in the peripheral layer of the outer root sheath of the infundibulum, the lower bulb and pigment matrix.

5. The epidermal melanocyte manifests a random distribution in the early differentiating epidermis from its time of epidermal entry up to six months fetal age although there appears a definite tendency for junctional localization at five months fetal age.

6. After six months fetal age epidermal melanocytes assume their permanent junctional distribution.

7. It is shown that embryonic human hair melanocytes and epidermal melanocytes undergo an essentially parallel process of differentiation and distribution.

8. The genesis and fate of high-level dopa and premelanin positive fetal melanocytes are discussed in relation to Langerhans cells.

9. Premelanin positive melanocytes greatly exceed dopa positive cells in number.

10. It is suggested that in the process of fetal melanocyte differentiation the melanosome formation by the Golgi apparatus and active tyrosinase synthesize by ribosomes can occur separately with the former preceding the latter.

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